Virchows Arch A (1987) 410:553–558

Virchows Archiv A

Megamitochondria as a diagnostic marker for alcohol induced centrilobular and periportal fibrosis in the liver

Jette Junge, Thomas Horn, and Per Christoffersen
Department of Pathology, University of Copenhagen, Hvidovre Hospital, DK-2650 Hvidovre, Denmark

Summary. One hundred and five biopsies with centrilobular and/or portal/periportal fibrosis of different aetiology were examined by light microscopy for the presence of megamitochondria (MM). Sixty eight patients had some daily alcohol intake, 37 did not. The incidence of MM in the group with daily alcohol consumption was 49% and in the group without only 5%. Two types of MM were identified. Type I MM (round to oval) were located mainly in zone 3 and type II (needle-shaped) mainly in zone 1. Both types were related to alcoholic liver disease.

Key words: Adaption – Alcoholic liver injury – Degenerative changes – Megamitochondria

Introduction

The pattern of collagen deposition (fibrosis) in the liver may be rather uniform independent of aetiology. Centrilobular fibrosis may be seen in connection with alcoholic liver disease (Edmonson et al. 1963), as sequelae after viral hepatitis (Boyer et al. 1970) and in patients with passive congestion (Castberg 1952). Periportal fibrosis may be a sequela of viral hepatitis (De Groote et al. 1968) or ascending cholangitis induced by choledocholithiasis (Poulsen et al. 1970) or by alcohol induced chronic pancreatitis (Poulsen et al. 1979).

Thus, markers are important in order to differentiate between fibrosis of differing aetiologies. Mallory bodies and alcoholic hepatitis are important light microscopical markers for alcohol induced liver lesions (Christoffersen et al. 1973). Megamitochondria (MM) in hepatocytes may also be such a marker but little attention has been centered on the occurrence and significance of MM.

Ultrastructural investigations have demonstrated morphological changes in mitochondria induced by alcohol both in experimental models (Rømert et al. 1983) and in human alcoholics (Kiessling et al. 1964; Petersen 1977; Svoboda et al. 1964). Mitochondria may, however, enlarge to sizes visible by light microscopy, (MM) and some studies have suggested that this change may be related to alcohol consumption (Bruguera et al. 1977; Stewart et al. 1982; Uchida et al. 1984; Yokoo et al. 1982).

According to the sparse literature, the incidence of MM in alcoholics varies from 25% (Yokoo et al. 1978) to as high as 93% (Stewart et al. 1982). Yokoo et al. (1978) found MM to be specific for alcoholic liver disease, but this has been disproven by others (Kiessling et al. 1964; Stewart et al. 1982; Uchida et al. 1984). Different types of MM (round (type I), elongated (type II)) can be identified (Bruguera et al. 1977; Iseri et al. 1971; Uchida et al. 1984). Whereas both types were related to alcoholic liver injury in one study (Yokoo et al. 1978), Uchida et al. (1984) only found type I MM to be related to alcoholic liver disease.

Thus, there are conflicting views on the occurrence and the significance of MM in alcoholic liver disease. In work in progress concerning early alcoholic liver damage we found MM to be easily recognizable and the present work reports results on both the occurrence and the diagnostic significance of MM.

Materials and methods

The material was selected in order to represent early alcoholic change and corresponding changes of non-alcoholic genesis, it comprises 105 liver biopsies from 105 different patients. Biop-

Offprint requests to: J. Junge at the above address

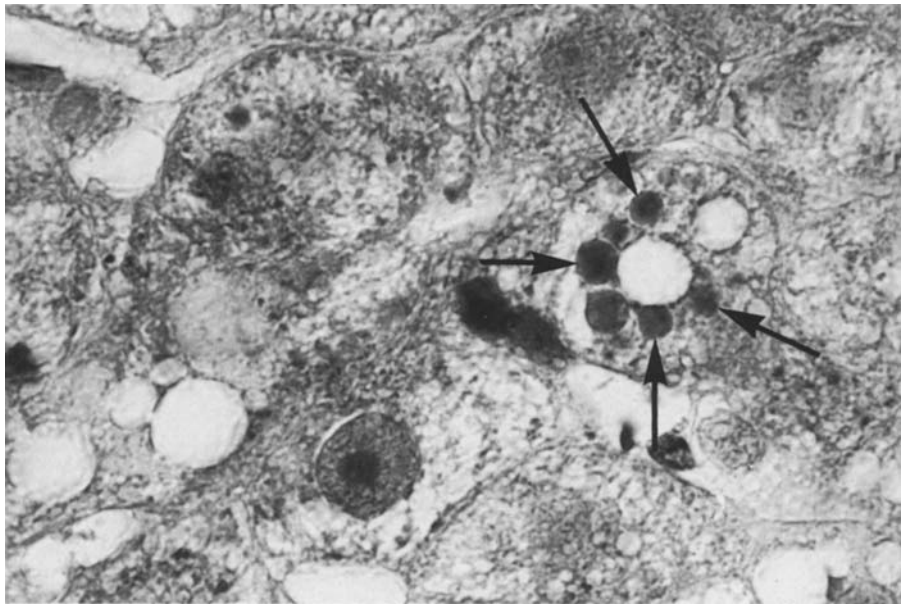

Fig. 1. Type I MM (*arrows*) in a hepatocyte also revealing microvesicular steatosis. Masson trichrome stain. Original magnification × 1,000

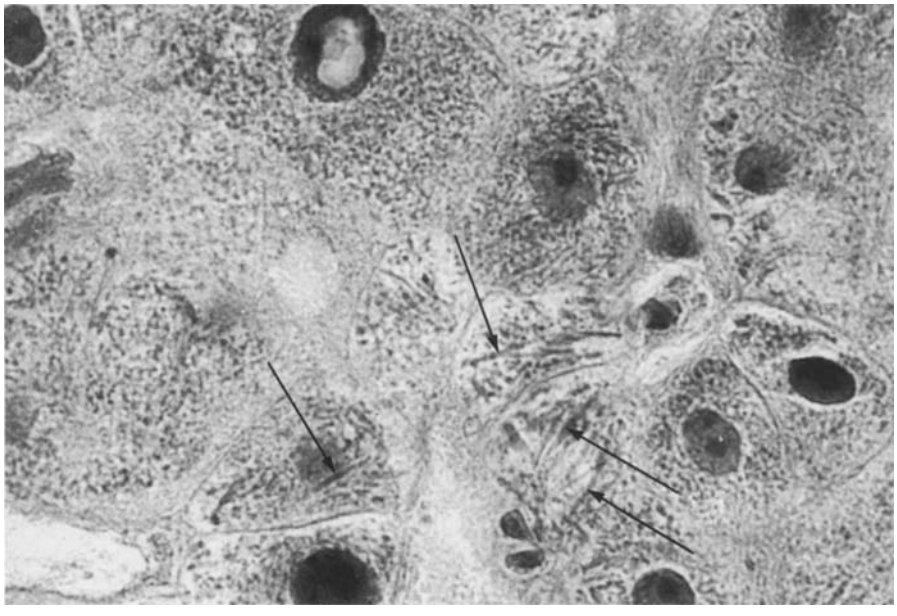

Fig. 2. Hepatocytes containing type II MM (arrows). Masson trichrome stain. Original magnification × 1,000

sies were selected retrospectively as consecutive biopsies with preserved architecture and either centrilobular or portal/periportal fibrosis. The material thus includes biopsies with predominatly parenchymal changes induced by alcohol, congestion or hepatitis (drugs and virus) and predominantly portal/periportal changes (pancreatitis or stones).

The biopsies were stained according to our routine procedure (Poulsen et al. 1979) and MM were defined as intracytoplasmic bright red structures visible in the Masson's trichrome stain and negative in PAS after diastase digestion (Bianchi et al. 1973; Uchida et al. 1984). Only elements larger than the nucleolus were registered as MM, and they were classified as type I (round to oval) or type II (needle-shaped).

The zonal distribution and the number of MM were registered. When only scattered hepatocytes in the total specimen contained MM this was registered as (+). MM in numerous hepatocytes in each acinus were registered as (+ + +), and (+ +) was noted when several hepatocytes contained MM in several acini but not in all. Furthermore, it was recorded whether MM occurred in normal hepatocytes or in cells with other cytoplasmic changes (microvesicular steatosis, macrovesicular steatosis or Mallory bodies).

The degree of fatty change was classified as slight, moderate or severe. Centrilobular fibrosis (perisinusoidal/pericellular or a mixture of both) was registered as absent or present in slight, moderate or severe degree.

Data concerning age, sex and alcohol consumption were obtained from the clinical records. The interval between admission to hospital and biopsy was registered.

The amount of alcohol intake was assessed based on inter-

Table 1. The main histological diagnosis and the occurrence of MM in 37 biopsies from patients without and 68 biopsies from patients with a daily alcohol comsumption

		No. of biopsies	Biopsies with MM			
			No.	Type I only	Type I+II	Type II only
Patients with no daily alcohol consumption (Group I)	Centrilobular fibrosis (chronic congestion)	7	1	–	–	1
	Centrilobular fibrosis (acute hepatitis)	18	1	–	1	–
	Centrilobular fibrosis	4	–	–	–	–
	Large duct obstruction	8	–	–	–	–
	Total	37	2	–	1	1
Patients with a daily alcohol consumption (Group II)	Centrilobular fibrosis	21	9	6	3	–
	Centrilobular fibrosis (alcoholic hepatitis)	18	12	7	5	–
	Centrilobular fibrosis (chronic congestion)	7	4	–	2	2
	Centrilobular fibrosis (acute hepatitis)	2	–	–	–	–
	Large duct obstruction	20	8	6	1	1
	Total	68	33	19	11	3

view in the clinical records and the patients were divided into four groups: *1*) no daily alcohol consumption, *2*) daily alcohol consumption of less than 60 g, *3*) between 60 g and 120 g and *4*) a daily alcohol consumption of more than 120 g.

Two biopsies revealing round MM and two biopsies revealing needle-shaped MM were deparaffinized in xylene, post-fixed in osmium tetroxide and then embedded in Epon. One μm thick sections for orientation were stained with Toluidine Blue. Thin sections were stained with uranyl acetate-lead citrate before examination in a JEOL 100C electron microscope.

Table 2A. Type of MM correlated to amount of daily alcohol intake

MM type	No alcohol (37)	<60 g (16)	60–120 g (32)	>120 g (20)
I	0	5	8	6
I+II	1	3	4	4
II	1	2	1	0

Table 2B. Number of MM correlated to daily alcohol intake. The number in parenthesis refers to the total number of biopsies in each group

MM number	No alcohol (37)	<60 g (16)	60–120 g (32)	>120 g (20)
+	0	4	7	8
++	1	4	2	2
+++	1	2	4	0

Results

In haematoxylin-eosin stained sections MM appeared as faint eosinophilic globules. They were, however, most easily identified in the Masson trichrome stain where they appeared bright-red either as round-oval structures (type I) (Fig. 1) or needle-shaped structures (type II) (Fig. 2). MM were observed in otherwise normal cells or in cells with microcytic fatty change.

Table 1 shows the main histological diagnosis and the occurrence of MM in liver biopsies from patients without (Group I) and 68 patients with a daily alcohol consumption (Group II). MM were found in two biopsies from Group I (5%) and in 49% of the biopsies from Group II (33/68).

MM type I alone was observed in 19 biopsies from Group II (patients with daily alcohol consumption) and type I and II together were found in 11 biopsies from Group II and in one from Group I, while MM type II alone were observed in three with a daily alcohol consumption (Group II) and in one without (Group I).

Concerning the acinar distribution, MM type I, when present alone, were almost exclusively located in zone 3 (18/19), and type II, when present alone, were located in zone 1 (3/4). With simultaneous occurrence of type I and II, six biopsies showed a diffuse distribution of both types, while the remaining six biopsies showed type I in zone 3 and type II in zone 1.

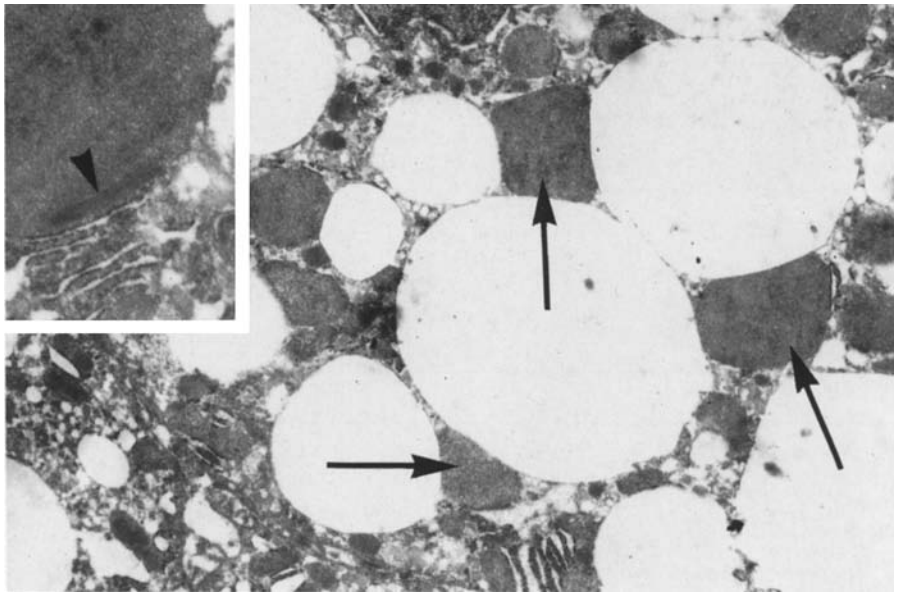

Fig. 3. Electron micrograph of type I MM (arrows) containing a finely granular material. Original magnification × 5,000. *Insert:* High magnification showing cristae-like structures (*arrow head*). Original magnification × 20,000

Fig. 4. Electron micrograph of type II MM. Cristae-like structures are marked with arrows. Original magnification × 10,000

The number of MM varied not only from cell to cell but also from area to area in the same biopsy. The type of MM and the number are listed and correlated to alcohol consumption in Table 2A and 2B. MM of both types occurred significantly more often ($p < 0.05$) in group II than in Group I. Although not statistically significant, MM occurred more often and in greater number in biopsies from patients with the lowest alcohol consumption. There was a significant difference between the occurrence in patients without daily alcohol consumption and in patients with low daily intake (less than 60 g/day) ($p < 0.001$).

Mallory bodies (with and without surrounding neutrophils) were not present in any biopsies from Group I but were registered in 37% of the biopsies from Group II (25/68). Twenty five of biopsies from Group II contained neither Mallory bodies nor MM, 10 contained only Mallory bodies, 15 both Mallory bodies and MM, and 18 only MM. Both types of changes occurred significantly more often in Group II than in Group I, but Mallory bodies occurred more often than MM in patients with heavy daily alcohol consumption.

Globules positive with PAS after diastase digestion were found in only one biopsy from a non-alcoholic.

Most of the biopsies (81%) from patients with

a daily alcohol consumption showed some degree of fatty changes, or of parenchymal fibrosis (pericellular and/or parisinusoidal) (81%).

In the group with a moderate or heavy daily alcohol consumption, 38% (26/68) of the biopsies showed moderate or severe steatosis while only 3% (2/68) in the group with a daily alcohol intake of less than 60 g/day revealed moderate or severe steatosis. MM occurred more often in biopsies with no or slight fatty change.

There was no correlation between the alcohol intake and the degree of parenchymal fibrosis, and there was no correlation between this fibrosis and the occurrence of MM. Nor was any relationship found between the occurrence of MM and age, sex, duration and abstinence.

By ultrastructural investigation both types of MM were identified. Type I contained a finely granular electron dense material (Fig. 3), and only occasionally cristae-like structures. Type II contained numerous, often longitudinally arranged cristae-like structures, some of which may represent paracrystalline inclusions (Fig. 4). Although ultrastructural preservation was not optimal, MM have shown the same characteristics in studies by others (Uchida et al. 1984).

Discussion

Liver fibrosis with centrilobular and portal/periportal locations is found as part of quite different disease entities. In order to obtain a more specific diagnosis with special emphasis on aetiological factors, other variables than the pattern of fibrosis have to be evaluated.

While the significance of Mallory bodies as a morphological marker for alcoholic aetiology has been thoroughly elucidated little interest has been focused on MM, even though the latter are easily recognizable structures by light microscopy. Using different staining for liver biopsies (Poulsen et al. 1979) there should normally be no confusion in differentiating MM from other cytoplasmic inclusions. In contrast to alpha-1-antitrypsin globules, MM are negative with PAS after digestion and, in the Masson Trichrome, typical Mallory bodies appear dark blue, smaller round Mallory bodies are greyish, while MM appear bright red. Ultrastructurally, we confirmed that the inclusions in question actually represented MM, and our findings pertaining to the morphology of both type I and II MM do not differ from others (Uchida et al. 1984).

In the present study, the incidences of MM in biopsies from patients with and without a daily alcohol consumption was 49% and 5%, respectively. In other investigations, the incidence among chronic alcoholics varied from 25% (Yokoo et al. 1978) to 93% (Stewart et al. 1982) and, among non-alcoholics from 0% (Yokoo et al. 1978) to 37% (Stewart et al. 1982). A comparison to our results is, however, impossible due to differences in the materials.

Like Yokoo et al. (1978) and Uchida et al. (1984), we found type I MM to be located mainly in centrilobular areas (zone 3) and type II in periportal areas (zone 1). The reason for this topographical distribution is unknown, but could be associated with differences in the zonal blood supply as described by Rappaport (1976) and/or with differences in hepatocellular maturity in zone 1 and 3 as reported by Zajicek et al. (1985).

The occurrence of MM, especially of type I, is diagnostic for alcohol induced changes and is a very sensitive marker in our material. Mallory bodies were present in 37% of the biopsies from patients with a daily alcohol consumption. Additionally, by documentation of MM, a further 26% of our biopsies could be allocated in the group of patients with a daily alcohol consumption. However, two biopsies from non-alcoholics also contained MM. This could be caused by false information given in the medical records, but is most probably an indication of a rather stereotypic manner of reaction mode of the hepatocytes. As is the case with Mallory bodies the formation of MM may be induced by several factors, but primarily by alcohol consumption.

It is noteworthy that MM were most common in the group of patients with a low alcohol consumption and with mild steatosis in the biopsies. This is in accordance with the results of Chedid et al. (1986) but in contrast with the fact that Mallory bodies are normally found among heavy drinkers and in biopsies showing significant steatosis. This difference may be due to difficulties in recognizing MM in hepatocytes with severe changes (fat droplets occupying the vast majority of the cytoplasm or Mallory bodies).

References

Bianchi L, Winckler K, Mihatsch M, Rohr, HP (1973) Mallory bodies and giant mitochondria – Two different structures in liver biopsies from alcoholics. Beitr Pathol 150:298–310

Boyer FL, Klatskin G (1970) Pattern of necrosis in acute viral hepatitis. Prognostic value of bridging (subacute hepatic necrosis). N Engl J Med 283:1063–1107

Bruguera M, Bertarn A, Bombi JA, Rodes J (1977) Giant mitochondria in hepatocytes. A diagnostic hint for alcoholic liver disease. Gastroenterology 73:1383–1387

Castberg T (1952) Vascular changes in chronic passive congestion of the liver. Acta Path Microbiol Scand 30:358–370

Chedid A, Mendenhall CL, Tosch T, Chen T, Rabin L, Carcia-Pont P, Goldberg SJ, Kierman T, Seeff LB, Sorrell M, Tamburro L, Weesner RE, Zetterman R (1986) Significance of megamitochondria in alcoholic liver disease. Gastroenterology 90:1858–1864

Christoffersen P, Eghøje K, Juhl E (1973) Mallory bodies in liver biopsies from chronic alcoholics. Scand J Gastroenterol 8:341–346

De Groote J, Desmet V, Gedigk P, Koch G, Popper H, Poulsen H, Scheuer PJ, Schmid M, Thaler H, Uehlinger E, Wepler W (1968) A classification of chronic hepatitis. Lancet ii 626–628

Edmonson HA, Peters RL, Reynolds TB, Kuzma OT (1963) Sclerosing hyalin necrosis of the liver in the chronic alcoholics: A recognizable clinical syndrom. Ann Int Med 59:646–673

Iseri OA, Gottlieb LS (1971) Alcoholic hyalin and megamitochondria as separate and distinct entities in liver diseases associated with alcoholism. Gastroenterology 60:1027–1035

Kiessling K-H, Lindgren L, Strandberg B, Tobé U (1964) Electron microscopic study of liver mitochondria from human alcoholics. Acta Med Scand 176,5:595–598

Petersen P (1977) Abnormal mitochondria in hepatocytes in human fatty liver. Acta Path Microbiol Scand Sect A 85:413–420

Poulsen H, Christoffersen P (1970) Histological changes in liver biopsies from patients with surgical bile duct disorders. Acta Path Microbiol Scand A 78:571–579

Poulsen H, Christoffersen P (1979) Atlas of liver biopsies. Munksgaard, Copenhagen, Denmark, p 186

Rappaport AM (1976) The microcirculatory acinar concept of normal and pathological hepatic structure. Beitr Pathol 157:215–243

Rømert P, Mathiessen ME (1983) Alcohol-induced injury of mitochondria in hepatocytes of mini-pig fetuses. Virch Arch [Pathol Anat] 399:299–305

Stewart RV, Cincsoy HP (1982) The significance of giant mitochondria in liver biopsies as observed by light microscopy. AJCP:293–298

Svoboda DJ, Manning RT (1964) Chronic alcoholism with fatty metamorphosis of the liver. Mitochondrial alterations in hepatic cells. Am J Pathol 44:645–652

Uchida T, Kronborg I, Peters RL (1984) Giant mitochondria in the alcoholic liver diseases – their identification, frequency and pathologic significance. Liver 4:29–38

Yokoo H, Singh SK, Hawasli AH (1978) Giant mitochondria in alcoholic liver disease. Arch Path Lab Med 102:213–224

Zajicek G, Oven R, Weinreb M (1985) The streaming liver. Liver 5:293–300

Accepted October 1986